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ORIGINAL ARTICLE

Hematinic deficiencies and anemia statuses in recurrent aphthous stomatitis patients with or without atrophic glossitis

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Background/Purpose: Some of recurrent aphthous stomatitis (RAS) patients had concomitant atrophic glossitis (AG). This study assessed whether RAS patients with AG (AG+/RAS patients) or without AG (AG−/RAS patients) had anemia and hematinic deficiencies and to evaluate whether RAS combined with AG or RAS itself was a significant factor causing anemia and hematinic deficiencies in AG+/RAS or AG−/RAS patients, respectively.
Methods: The mean corpuscular volume (MCV) and mean blood hemoglobin (Hb), iron, vitamin B12, and folic acid levels were measured and compared between any two of three groups of 160 AG+/RAS patients, 195 AG-/RAS patients, and 355 healthy control subjects.
Results: Both AG+/RAS and AG−/RAS patients had significantly lower mean Hb, iron, and vitamin B12 levels as well as significantly greater frequencies of Hb, iron, vitamin B12, and folic acid deficiencies than healthy control subjects. Moreover, AG+/RAS patients had significantly lower mean Hb and serum iron level (for women only) and significantly greater frequencies of Hb and iron deficiencies than AG−/RAS patients. Of 69 anemia AG+/RAS patients, 30 (43.5%) had normocytic anemia and 23 (33.3%) had iron deficiency anemia. Of 38 anemia AG−/RAS patients, 26 (68.4%) had normocytic anemia and 5 (13.2%) had iron deficiency anemia.
Conclusion: We conclude that some of AG+/RAS or AG−/RAS patients do have anemia and hematinic deficiencies and AG+/RAS patients do have severer anemia statuses and iron

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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deficiency than AG−/RAS patients. RAS combined with AG or RAS itself does play a significant role in causing anemia and hematinic deficiencies in AG+/RAS or AG−/RAS patients, respectively.

Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disease characterized by recurrent and painful ulcerations on the nonkeratinized oral mucosae such as labial, buccal, alveolar, and ventral tongue mucosae. The prevalence of RAS ranges from 5% to 20% depending on the population evaluated.[1] In Taiwan, the prevalence of RAS is 10.5% in the general population.[2]

Although several etiological factors have been proposed, the exact causes of RAS are still not very clear.[3] The results of previous studies on tissue infiltrated mononuclear cells favor the role of cell-mediated cytotoxicity in the immunopathogenesis of RAS.[4] In addition to immune dysregulation, multiple nutritional deficiencies including deficiencies of vitamins B1, B2, B6, and B12, folate, iron, and ferritin are considered to be the possible etiologies of RAS.[4] Our previous studies showed that 57 (20.9%), 55 (20.1%), 13 (4.8%) and 7 (2.6%) of 273 RAS patients as well as 39 (22.2%), 47 (26.7%), 13 (7.4%) and 3 (1.7%) of 176 atrophic glossitis (AG) patients have deficiencies of Hb, iron, vitamin B12, and folic acid, respectively.[4,5] Our clinical experience revealed that a portion of RAS patients may have concomitant AG. Thus, it was interesting to know whether RAS patients with AG (AG+/RAS patients) had severer anemia status and hematinic deficiencies than RAS patients without AG (AG−/RAS patients).

In our oral mucosal disease clinic, patients with AG, burning mouth syndrome (BMS), oral lichen planus (OLP), RAS, oral submucous fibrosis (OSF), and other oral mucosal diseases are frequently encountered.[4–23] For AG, BMS, OLP, RAS and OSF patients, complete blood count, serum iron, vitamin B12, folic acid, homocysteine, and anti-gastric parietal cell antibody (GPCA) levels are frequently examined to assess whether these patients have anemia, hematinic deficiencies, and serum GPCA positivity.[4–20] In this study, we collected 355 RAS patients including 160 AG+/RAS and 195 AG−/RAS patients. Their complete blood counts as well as serum iron, vitamin B12, folic acid, homocysteine and GPCA levels were examined and compared with the corresponding data of 355 age- and sex-matched healthy control subjects. The purposes of this study were to study the anemia statuses and hematinic deficiencies in these 160 AG+/RAS and 195 AG−/RAS patients, to assess whether AG+/RAS patients had severer anemia status and hematinic deficiencies than AG−/RAS patients, to evaluate whether AG−/RAS patients still had anemia and hematinic deficiencies, to find out what were the common types of anemia in AG+/RAS and AG−/RAS patients, and to evaluate whether RAS combined with AG or RAS itself was a significant factor that caused anemia and hematinic deficiencies in AG+/RAS patients or AG−/RAS patients, respectively.

Materials and methods

Subjects

In this study, 160 (39 men and 121 women, age range 18—90 years, mean 55.7 ± 15.8 years) AG+/RAS patients and 195 (67 men and 128 women, age range 18—90 years, mean 50.4 ± 15.6 years) AG−/RAS patients were collected in the oral mucosal disease clinic of National Taiwan University Hospital (NTUH). For comparisons, 355 age-(±2 years of each patient's age) and sex-matched healthy control subjects (106 men and 249 women, age range 20—89 years, mean age 53.1 ± 14.7 years) were included in this study. All RAS patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry, NTUH from July 2007 to July 2016. Patients were diagnosed as having RAS when they had at least one episode of oral ulcerations on movable oral mucosa per month since childhood.[4] In this study, 160 AG+/RAS patients had concomitant partial or complete AG which was defined as partial or complete absence or flattening of filiform papillae on the dorsal surface of the tongue, respectively.[5] In contrast, 195 AG−/RAS patients did not have either partial or complete AG. RAS patients with betel quid chewing habit or autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid) were excluded. Moreover, patients with traumatic ulcers or with aphthous-like ulcers associated with systemic disorders including Behcet's syndrome, celiac disease, gluten-sensitive enteropathy, inflammatory bowel diseases, human immunodeficiency virus infection, and cyclic neutropenia were also excluded.[24] In addition, RAS patients with serum creatinine concentrations indicative of renal dysfunction (i.e., men, >131 μmol/L; women, >115 μmol/L), and who reported a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries were also excluded.[25] Healthy control subjects had either dental caries, pulpal disease, malocclusion, or missing of teeth but did not have any oral mucosal or systemic diseases. None of the RAS patients had taken any prescription medication for RAS or AG at least 3 months before entering the study.

The blood samples were drawn from all RAS patients and healthy control subjects for measurement of complete blood count, serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels. All RAS patients and healthy control subjects signed the informed consents before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH.

Determination of complete blood count and serum iron, vitamin B12, folic acid and homocysteine concentrations

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by the routine tests performed in the Department of Laboratory Medicine of NTUH as described previously.[4–17] This study defined the Hb and hematinic deficiencies according to the World Health Organization (WHO) criteria. Thus, men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[15,26] Patients with serum iron level $<$60 μg/dL,[27] vitamin B12 level $<$200 pg/mL[25] or folic acid level $<$4 ng/mL[28] were defined as having iron, vitamin B12 or folic acid deficiency, respectively. Moreover, patients with the serum homocysteine level $>$12.6 μM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having abnormally high homocysteine level.

Determination of serum GPCA level

The method of determination of serum GPCA level in our RAS patients and healthy control subjects have been described in our previous studies.[5–8] The serum GPCA level was measured to confirm whether RAS patients had pernicious anemia.

Statistical analysis

Comparisons of the mean MCV and mean blood levels of Hb, iron, vitamin B12, folic acid and homocysteine between AG+/RAS patients or AG−/RAS patients and healthy control subjects and between AG+/RAS patients and AG−/RAS patients were performed by Student's *t*-test. The differences in frequency of Hb, iron, vitamin B12 or folic acid deficiency, or frequency of abnormally high blood homocysteine level between AG+/RAS patients or AG−/RAS patients and healthy control subjects and between AG+/RAS patients and AG−/RAS patients were compared by chi-square test. Comparisons of mean MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between any two of the three groups of AG+/RAS patients or AG−/RAS patients with macrocytic, normocytic or microcytic RBCs were performed by Student's *t*-test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The mean MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 160 AG+/RAS patients, 195 AG−/RAS patients, and 355 healthy control subjects are shown in Table 1. Because men usually had higher blood levels of Hb and iron than women, these two mean levels were calculated separately for men and women. We found significantly lower mean Hb (for men and women, both *P*-values $<$ 0.001), iron (for men, $P = 0.001$; for women, $P < 0.001$), and vitamin B12 ($P = 0.030$) levels as well as significantly higher mean blood homocysteine level ($P = 0.003$) in AG+/RAS patients than in healthy control subjects (Table 1). Moreover, we found a significantly lower mean Hb (for men and women, both *P*-values $<$ 0.001), and iron level (for women only, $P = 0.039$) and a significantly higher mean blood homocysteine level ($P = 0.034$) in AG+/RAS patients than in AG−/RAS patients. Furthermore, AG−/RAS patients also had significantly lower mean Hb (for men and women, both *P*-values $<$ 0.001), MCV ($P < 0.001$), iron (for men, $P = 0.035$; for women, $P < 0.001$), vitamin B12 ($P = 0.053$, marginal significance), and folic acid levels ($P = 0.002$) than healthy control subjects (Table 1).

We also found significantly greater frequencies of Hb ($P < 0.001$), iron ($P < 0.001$), vitamin B12 ($P < 0.001$), and folic acid ($P = 0.014$) deficiencies and of high blood homocysteine level ($P < 0.001$) in AG+/RAS patients than in healthy control subjects (Table 2). There were also significantly greater frequencies of Hb ($P < 0.001$) and iron ($P = 0.001$) deficiencies in AG+/RAS patients than in AG−/RAS patients. Moreover, we also demonstrated significantly greater frequencies of Hb, iron, vitamin B12, and folic acid deficiencies (all *P*-values $<$ 0.001) and of high blood homocysteine level in AG−/RAS patients than in healthy control subjects (Table 2).

In this study, 69 (43.1%) of 160 AG+/RAS patients and 38 (19.5%) of 195 AG−/RAS patients were diagnosed as having anemia according to the WHO criteria (Table 3).[15,26] The different anemia types in 69 anemic AG+/RAS patients and in 38 anemic AG−/RAS patients are described in detail in Table 3. In this study, PA was diagnosed as having MCV $\geqq$ 100 fl, vitamin B12 $<$ 200 pg/mL, and serum GPCA positivity,[12–14] iron deficiency anemia as having MCV $<$80 fl and iron $<$60 μg/dL,[15,26,27] and thalassemia trait as having MCV $<$74 fl, RBC count $>5.0 \times 10^{12}$/L, and Mentzer index (MCV/RBC) $<$ 13.[18] By these definitions, of 69 anemic AG+/RAS patients, 3 had pernicious anemia, 6 had macrocytic anemia, 30 had normocytic anemia, 23 had iron deficiency anemia, 5 had thalassemia tract, and 2 had microcytic anemia but did not have iron deficiency or thalassemia tract. Moreover, of 38 anemic AG−/RAS patients, one had pernicious anemia, 2 had macrocytic anemia, 26 had normocytic anemia, 5 had iron deficiency anemia, 3 had thalassemia tract, and one had microcytic anemia but did not iron deficiency or thalassemia tract.

When 160 AG+/RAS patients were further divided into three groups: group 1 (10 patients with MCV $\geqq$ 100 fl), group 2 (117 patients with MCV between 80 fl and 99.9 fl), and group 3 (33 patients with MCV $<$ 80 fl), we found that group 1 patients had significantly lower mean Hb level (for men only, $P = 0.021$), higher MCV ($P < 0.001$), higher mean serum iron level (for men only, $P = 0.040$), lower mean serum vitamin B12 ($P < 0.001$), higher mean folic acid level ($P = 0.003$), and higher mean serum homocysteine level ($P < 0.001$) than group 2 patients (Table 4). Moreover, group 3 patients had significantly lower mean Hb level (for women only, $P < 0.001$), lower MCV ($P < 0.001$), and lower mean serum iron level (for women only, $P < 0.001$) than group 2 patients (Table 4). In addition, group 1 patients had higher mean Hb level (for women only, $P = 0.055$, marginal significance) and significantly higher MCV ($P < 0.001$), lower mean serum vitamin B12 level ($P < 0.001$), higher mean serum folic acid

Table 1 Comparisons of the mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid and homocysteine between any two of the three groups of 160 atrophic glossitis-positive recurrent aphthous stomatitis (AG+/RAS) patients, 195 AG-negative RAS (AG−/RAS) patients, and 355 healthy control subjects.

Group	Hb (g/dL)		MCV (fl)	Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
	Men	Women		Men	Women			
AG+/RAS patients (n = 160)	12.9 ± 1.8 (n = 39)	12.4 ± 1.5 (n = 121)	87.0 ± 9.9	83.6 ± 43.4 (n = 39)	76.7 ± 35.5 (n = 121)	620.3 ± 290.1	13.7 ± 6.9	10.6 ± 12.1
P-value[a]	<0.001	<0.001	<0.001	0.001	<0.001	0.030	0.399	0.003
P-value[b]	<0.001	<0.001	0.156	0.150	0.039	0.724	0.098	0.034
AG−/RAS patients (n = 195)	14.6 ± 1.3 (n = 67)	13.0 ± 1.2 (n = 128)	88.3 ± 7.3	94.1 ± 30.8 (n = 67)	85.5 ± 31.4 (n = 128)	630.7 ± 263.7	12.5 ± 6.7	8.5 ± 6.0
P-value[a]	<0.001	<0.001	<0.001	0.035	<0.001	0.053	0.002	0.775
Healthy control subjects (n = 355)	15.2 ± 0.7 (n = 106)	13.6 ± 0.7 (n = 249)	90.7 ± 3.7	103.7 ± 27.6 (n = 106)	99.0 ± 28.3 (n = 249)	673.7 ± 240.8	14.2 ± 5.9	8.6 ± 2.0

[a] Comparisons of parameters between 160 AG+/RAS patients or 195 AG−/RAS patients and 355 healthy control subjects.
[b] Comparisons of parameters between 160 AG+/RAS patients and 195 AG−/RAS patients.

Table 2 Comparisons of frequencies of hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and frequency of abnormally high blood homocysteine level between any two of the three groups of 160 atrophic glossitis-positive recurrent aphthous stomatitis (AG+/RAS) patients, 195 AG-negative RAS (AG−/RAS) patients, and 355 healthy control subjects.

	Hb deficiency (Men < 13 g/dL, Women < 12 g/dL)	Iron deficiency (<60 μg/dL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	High homocysteine level (>12.6 μM)
AG+/RAS patients (n = 160)	69 (43.1%)	57 (35.6%)	19 (11.9%)	4 (2.5%)	24 (15.0%)
P-value[a]	<0.001	<0.001	<0.001	0.014	<0.001
P-value[b]	<0.001	0.001	0.129	0.592	0.065
AG−/RAS patients (n = 195)	38 (19.5%)	39 (20.0%)	13 (6.7%)	8 (4.1%)	16 (8.2%)
P-value[a]	<0.001	<0.001	<0.001	<0.001	0.001
Healthy control subjects (n = 355)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (2.0%)

[a] Comparisons of parameters between 160 AG+/RAS patients or 195 AG−/RAS patients and 355 healthy control subjects.
[b] Comparisons of parameters between 160 AG+/RAS patients and 195 AG−/RAS patients.

level ($P < 0.001$), and higher mean serum homocysteine level ($P < 0.001$) than group 3 patients (Table 4).

We also divided 195 AG−/RAS patients into three groups: group 1 (3 patients with MCV ≧ 100 fl), group 2 (174 patients with MCV between 80 fl and 99.9 fl), and group 3 (18 patients with MCV < 80 fl). Because there was only one male patient in group 1, the Hb and iron data were not compared between group 1 and group 2 or group 3 male patients. We found that group 1 patients had significantly lower mean Hb level (for women only, $P = 0.003$), higher MCV ($P < 0.001$), higher mean serum iron level (for women only, $P = 0.024$), higher mean serum folic acid level ($P = 0.056$, marginal significance), and higher mean serum homocysteine level ($P < 0.001$) than group 2 patients (Table 5). Moreover, group 3 patients had significantly lower mean Hb level (for men, $P = 0.009$; for women, $P < 0.001$), lower MCV ($P < 0.001$), lower mean serum iron level (for women only, $P = 0.022$), and lower mean serum folic acid level ($P = 0.014$) than group 2 patients (Table 5). In addition, group 1 patients had significantly higher MCV ($P < 0.001$), higher mean serum iron level (for women only, $P = 0.059$, marginal significance), higher mean serum folic acid level ($P < 0.001$), and higher mean serum homocysteine level ($P = 0.005$) than group 3 patients (Table 5).

Discussion

Our previous study demonstrated that 273 RAS patients have significantly greater frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and of high serum homocysteine level than 273 age/sex-matched healthy control subjects.[4] However, when the 273 RAS patients are further divided into 32 major-typed RAS and 241 minor-typed RAS patients, there are no significant differences in the frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and of high serum homocysteine level between the major-typed RAS and minor-typed RAS patients.[4] This study also found that both AG+/RAS and AG−/RAS patients had significantly greater frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and of high serum homocysteine level than healthy control subjects. Moreover, AG+/RAS had significantly greater frequencies of Hb and serum iron deficiencies than AG−/RAS patients. These findings indicate that a significant percentage of either AG+/RAS or AG−/RAS patients do have anemia and hematinic deficiencies and AG+/RAS patients do have severer anemia status and iron deficiency than AG−/RAS patients. The findings from our two RAS studies also suggest that both diseases of AG and RAS play

Table 3 Anemia types of 69 of 160 atrophic glossitis-positive recurrent aphthous stomatitis (AG+/RAS) patients and 38 of 195 AG-negative RAS (AG−/RAS) patients.

Anemia type	Patient number (%)				
	Patient number (%)	MCV	Vitamin B12 deficiency (<200 g/mL)	Iron deficiency (<60 μg/dL)	Folic acid deficiency (<4 ng/mL)
AG+/RAS patients (n = 160)					
Pernicious anemia	3 (4.3)	≧100 fl	3 (100.0)	0 (0.0)	0 (0.0)
Macrocytic anemia	6 (8.7)	≧100 fl	6 (100.0)	1 (16.7)	0 (0.0)
Normocytic anemia	30 (43.5)	80−99.9 fl	2 (6.7)	17 (56.7)	1 (3.3)
Iron deficiency anemia	23 (33.3)	<80 fl	2 (8.7)	23 (100.0)	3 (13.0)
Thalassemia trait	5 (7.2)	<74 fl	0 (0.0)	0 (0.0)	0 (0.0)
Microcytic anemia	2 (2.9)	<80 fl	1 (50.0)	0 (0.0)	0 (0.0)
Total	69 (100.0)		14 (20.3)	41 (59.4)	4 (5.8)
AG−/RAS patients (n = 195)					
Pernicious anemia	1 (2.6)	≧100 fl	1 (100.0)	0 (0.0)	0 (0.0)
Macrocytic anemia	2 (5.3)	≧100 fl	1 (50.0)	0 (0.0)	0 (0.0)
Normocytic anemia	26 (68.4)	80−99.9 fl	3 (11.5)	13 (50.0)	2 (7.7)
Iron deficiency anemia	5 (13.2)	<80 fl	1 (20.0)	5 (100.0)	0 (0.0)
Thalassemia trait	3 (7.9)	<74 fl	0 (0.0)	0 (0.0)	0 (0.0)
Microcytic anemia	1 (2.6)	<80 fl	0 (0.0)	0 (0.0)	1 (100.0)
Total	38 (100.0)		6 (15.8)	18 (47.4)	3 (7.9)

Table 4 The mean corpuscular volume (MCV), mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 160 atrophic glossitis-positive recurrent aphthous stomatitis (AG+/RAS) patients including 10 with MCV ≧ 100 fl (group 1), 117 with MCV between 80 fl and 99.9 fl (group 2), and 33 with MCV < 80 fl (group 3).

	AG+/RAS patients (n = 160)					
	Group 1 MCV ≧ 100 fl (n = 10)	*P*-value* (Group 1 vs. 2)	Group 2 MCV 80-99.9 fl (n = 117)	*P*-value* (Group 2 vs. 3)	Group 3 MCV < 80 fl (n = 33)	*P*-value* (Group 1 vs. 3)
	Mean ± SD		Mean ± SD		Mean ± SD	
Hb (g/dL)						
Men	11.6 ± 0.8 (n = 7)	0.021	13.4 ± 1.9 (n = 26)	0.061	11.8 ± 1.3 (n = 6)	0.740
Women	12.0 ± 0.4 (n = 3)	0.200	12.9 ± 1.2 (n = 91)	<0.001	10.7 ± 1.1 (n = 27)	0.055
MCV (fl)	107.5 ± 7.2	<0.001	89.4 ± 3.9	<0.001	72.2 ± 6.1	<0.001
Iron (μg/dL)						
Men	116.4 ± 64.0 (n = 7)	0.040	78.9 ± 33.2 (n = 26)	0.405	65.5 ± 43.1 (n = 6)	0.127
Women	62.0 ± 12.1 (n = 3)	0.200	85.5 ± 31.3 (n = 91)	<0.001	48.6 ± 35.9 (n = 27)	0.531
Vitamin B12 (pg/mL)	156.9 ± 14.8	<0.001	660.5 ± 265.6	0.433	618.2 ± 299.0	<0.001
Folic acid (ng/mL)	20.3 ± 5.5	0.003	13.6 ± 6.8	0.232	12.0 ± 6.6	<0.001
Homocysteine (μM)	40.2 ± 32.0	<0.001	8.5 ± 4.3	0.472	9.3 ± 8.9	<0.001

* Comparisons of MCV, mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between groups 1 and 2, between groups 2 and 3, and between groups 1 and 3 by Student's *t*-test.

significant roles in causing anemia and hematinic deficiencies (especially iron deficiency) in AG+/RAS patients, and RAS itself does play an important role in causing anemia and hematinic deficiencies in AG−/RAS patients.

It is interesting to understand the cause-and-effect relation between anemia or hematinic deficiencies and RAS. Our previous RAS study and this study showed that Hb, serum iron, vitamin B12, and folic acid deficiencies can be detected in 20−43%, 19−36%, 4−12%, and 2−6% of RAS patients, respectively, depending on different subtypes of RAS patients.[4] In addition, a high homocysteine level was found in 7−15% of different subtypes of RAS patients.[4] Our

Table 5 The mean corpuscular volume (MCV), mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 195 atrophic glossitis-negative recurrent aphthous stomatitis (AG−/RAS) patients including 3 with MCV ≧ 100 fl (group 1), 174 with MCV between 80 fl and 99.9 fl (group 2), and 18 with MCV < 80 fl (group 3).

	AG−/RAS patients (n = 195)					
	Group 1 MCV ≧ 100 fl (n = 3)	*P*-value* (Group 1 vs. 2)	Group 2 MCV 80−99.9 fl (n = 174)	*P*-value* (Group 2 vs. 3)	Group 3 MCV < 80 fl (n = 18)	*P*-value* (Group 1 vs. 3)
	Mean ± SD		Mean ± SD		Mean ± SD	
Hb (g/dL)						
Men	11.7 (n = 1)	ND	14.8 ± 1.2 (n = 62)	0.009	13.1 ± 1.6 (n = 4)	ND
Women	10.8 ± 0.8 (n = 2)	0.003	13.2 ± 1.1 (n = 112)	<0.001	11.9 ± 1.1 (n = 14)	0.200
MCV (fl)	107.0 ± 1.3	<0.001	89.8 ± 3.9	<0.001	70.7 ± 6.9	<0.001
Iron (μg/dL)						
Men	77 (n = 1)	ND	95.6 ± 31.1 (n = 62)	0.211	75.5 ± 25.7 (n = 4)	ND
Women	134.0 ± 60.8 (n = 2)	0.024	87.0 ± 28.4 (n = 112)	0.022	67.3 ± 41.3 (n = 14)	0.059
Vitamin B12 (pg/mL)	416.7 ± 424.4	0.140	643.2 ± 260.1	0.131	545.8 ± 250.3	0.459
Folic acid (ng/mL)	20.3 ± 6.4	0.056	12.8 ± 6.7	0.014	8.8 ± 4.5	<0.001
Homocysteine (μM)	28.2 ± 28.5	<0.001	8.2 ± 4.8	0.797	8.5 ± 3.5	0.005

ND = not done.

* Comparisons of MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between groups 1 and 2, between groups 2 and 3, and between groups 1 and 3 by Student's *t*-test.

previous RAS study also described deficiencies of vitamins B1, B2, B6 and B12, folate, iron, ferritin and Hb in a portion of RAS patients.[4] Iron deficiency causes microcytic anemia[27] and deficiencies of vitamin B12 and folic acid lead to macrocytic anemia.[25,29] RAS patients with anemia and lower Hb levels have reduced capacity of the blood to carry oxygen to oral mucosa, finally resulting in atrophy of oral mucosa. In addition, iron is essential to the normal functioning of oral epithelial cells[30] and both vitamin B12 and folic acid play important roles in DNA synthesis and cell division.[25,29] Oral epithelial cells have a high turnover rate. Therefore, deficiencies of iron, vitamin B12 and folic acid may result in oral epithelial atrophy. Atrophic oral epithelium in hematinic-deficient patients may explain why some patients with deficiencies of hematinics are prone to have RAS. Furthermore, high blood homocysteine level may result in an elevated frequency of thrombosis in the feeding arterioles that supply the oral epithelial cells.[31–35] This in turn leads to a breakdown of oral epithelium and finally produces an oral ulceration. Moreover, replacement therapy with hematinics for RAS patients with deficiencies of corresponding hematinics can result in a significant clinical improvement or at least a reduction in frequency and severity of their oral aphthous ulcers.[36–39] Taken these together, anemia, hematinic deficiencies, and high blood homocysteine levels can decrease oral epithelial barrier and thus increase the frequency of RAS occurrence. Moreover, Hb and hematinic deficiencies and high blood homocysteine level may be the causes of RAS.

We further explain why RAS alone or RAS combined with AG may be the important factors causing anemia and hematinic deficiencies in RAS patients with or without AG. The results of a previous diet history questionnaire study indicate a significantly lower daily intake of vitamin B12 and folate in minor-typed RAS patients than in control subjects.[40] By definition, the major-typed RAS patients often have severer and larger recurrent oral ulcerations than minor-typed RAS patients.[4] These severer ulcerative lesions may cause burning sensation and pain of the lesional oral mucosae when the patients eat salty and spicy food stuffs. The eating difficulty may result in reduced food intake that in turn leads to anemia and hematinic deficiencies in a certain percentage of our RAS patients. Regarding the AG, our previous study showed burning sensation of the tongue, dry mouth, numbness of oral mucosa, and dysfunction of taste in 100%, 79%, 57.4%, and 27.8% of 176 AG patients, respectively.[5] Burning sensation of the tongue, dry mouth, numbness of oral mucosa, and dysfunction of taste all may interfere with the eating and swallowing function of AG patients. This can further explain why AG patients do have anemia and hematinic deficiencies. Indeed, Hb, iron, vitamin B12, and folic acid deficiencies and high blood homocysteine level can be found in 22.2%, 26.7%, 7.4%, 1.7% and 21.6% of our 176 AG patients, respectively.[4] Furthermore, supplement treatments with vitamins BC capsules plus corresponding deficient hematinics (vitamin B12, folic acid or iron) or with vitamin BC capsules alone can not only reduce the high serum homocysteine levels to significantly lower levels but also eliminate oral symptoms and signs to a normal oral condition in AG patients.[19] The successful clinical outcomes for AG patients treated with deficient multiple vitamin and

hematinic supplementations suggest that multiple vitamin and hematinic deficiencies are the possible causes of AG.[19] Moreover, the RAS- and AG-induced reduction of food stuff intake can explain why RAS combined with AG may be the causes of anemia and hematinic deficiencies in AG+/RAS patients, and RAS itself may be an important factor causing anemia and hematinic deficiencies in AG−/RAS patients.

In this study, 69 (43.1%) of the 160 AG+/RAS patients and 38 (19.5%) of 195 AG−/RAS patients had anemia.[15,26] Six types of anemia including pernicious, macrocytic, normocytic, iron deficiency, or microcytic anemia and thalassemia trait were detected in RAS patients. We also found that 4 of 4 pernicious anemia patients and 7 of 8 macrocytic anemia patients (one patient had concomitant iron deficiency) had vitamin B12 but not folic acid deficiency, suggesting that these two types of anemia are predominantly due to vitamin B12 deficiency. Of 56 normocytic anemia patients, 30 (53.6%) had iron deficiency, 5 (8.9%) had vitamin B12 deficiency, and 3 (5.4%) had folic acid deficiency, indicating that normocytic anemia in our RAS patients are predominantly due to iron deficiency and secondarily due to vitamin B12 and folic acid deficiencies. In our 28 iron deficiency anemia patients, 3 had concomitant vitamin B12 deficiency and another 3 had folic acid deficiency. None of our 8 thalassemia trait patients had vitamin B12, iron or folic acid deficiency. The three microcytic anemia patients did not have iron deficiency, but one of them had vitamin B12 deficiency and another one had folic acid deficiency.

Of the 160 AG+/RAS patients, 10 (6.3%, group 1 patients) produced macrocytic RBCs, 117 (73.1%, group 2 patients) normocytic RBCs, and 33 (20.6%, group 3 patients) microcytic RBCs. Compared to group 2 patients, both groups 1 and 3 patients had relatively severe anemia, group 3 patients tended to have relatively low serum iron level, group 1 patients usually had low serum vitamin B12 level and significantly high blood homocysteine level, and group 3 patients usually had no vitamin B12 and folic acid deficiencies as well as no abnormally high blood homocysteine level. A similar tendency was also observed in 195 AG−/RAS patients including 3 macrocytosis (group 1), 174 nomocytosis (group 2), and 18 microcytosis (group 3) AG−/RAS patients.

We conclude that a significant percentage of either AG+/RAS or AG−/RAS patients do have anemia and hematinic deficiencies and AG+/RAS patients do have severer anemia status and iron deficiency than AG−/RAS patients. Both diseases of AG and RAS play significant roles in causing anemia and hematinic deficiencies (especially iron deficiency) in AG+/RAS patients, and RAS itself does play an important role in causing anemia and hematinic deficiencies in AG−/RAS patients. Normocytic anemia and iron deficiency anemia are two common types of anemia in RAS patients including AG+/RAS and AG−/RAS patients.

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